Recent Advances in Electrochemical Glyco-biosensing

Abstract

Biosensors that use electrochemical transduction mechanisms have made advances into the field of glycan analysis in the last 4 years. These glyco-biosensor assays offer simple, rapid, sensitive and economical approaches to the measurement need for rapid glycan analysis for biomarker detection, cancer and disease diagnostics and bioprocess monitoring of therapeutic glycoproteins. Although the prevalent methods of glycan analysis provide detailed identification and structural analysis of glycan species, there are significantly fewer rapid glycan assays available for diagnostic and screening applications. Here we review instances in which glyco-biosensors have been used for glycan analysis using a variety of electrochemical transduction mechanisms (e.g. amperometric, potentiometric, impedimetric and voltammetric), selective binding agents (e.g. lectins and antibodies) and redox species (e.g. enzyme substrates, inorganic, and nanomaterial).

Keywords: Glycan, glycosylation, protein, biosensor, electrochemical, lectin, electrochemical impedance spectroscopy, differential pulse voltammetry, cyclic voltammetry, pulsed amperometric detection, potentiometry, square wave voltammetry, biomarkers, bacteria

1. Introduction

Glycosylation is the process by which a glycan (i.e. saccharide or carbohydrate) is added to a non-glycan moiety (e.g. protein) and is the most common post translational modification of proteins [1]. The glycoprofile of a protein profoundly influences structure, function, stability, and serum half-life

which in turn affects many biological processes. Glycosylation plays a role in cell-cell interactions and has been found to be linked to several disease states, including infection, genetic disorders, and cancer [2-4]. In the case of cancer, abnormal protein glycosylation has been linked to early tumor cell growth and proliferation; therefore glycan based biomarkers have been sought for early detection of cancer and other disease states [3, 5-9].

Protein glycans are classified as either *N*-linked or *O*-linked (Figure 1). *N*-linked glycans are attached to the peptide at an Asn-X-Ser/Thr sequence site, where X ≠ proline, and share a common branched trimannosyl core. There are three *N*-glycan subtypes: *high-mannose* which have mannose residues attached to the mannose core, *complex* that do not contain terminal mannose residues but have complex branching and *hybrid* which contain both mannose residues and complex branching. *O*-linked glycans tend to be less complex (i.e. linear), they do not share a common single core, and they attach through serine or threonine residues (GalNAcα1-O-Ser/Thr). The seven monosaccharides found in human glycoprotein are mannose (Man), glucose (Glc), fucose (Fuc), galactose (Gal), *N*-acetylgalactosamine (GalNAc), N-acetylglucosamine (GlcNAc) and sialic acid (SA) or neuraminic acid (NeuNAc).

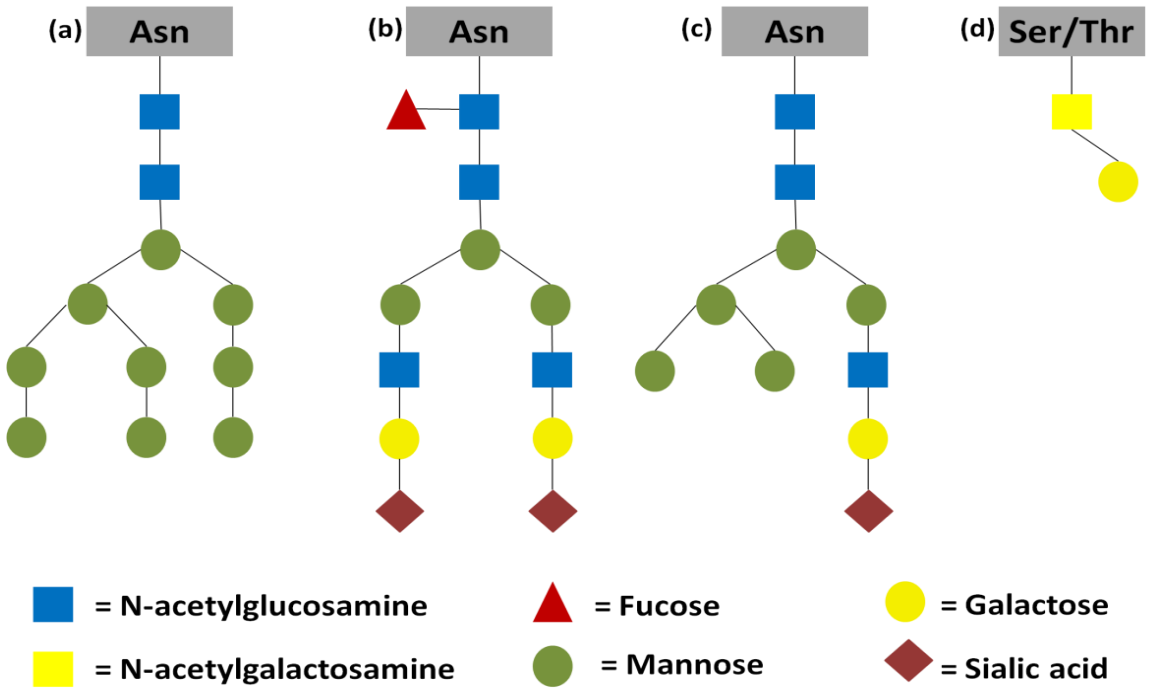

Figure 1. Oligosaccharide structure of the most common N-linked (a-c) and O-linked (d) glycans, including (a) high-mannose, (b) complex, and (c) hybrid glycans, and (d) the TF antigen.

The variation in glycosylation has also been shown to influence the biological activity (efficacy) and immunogenicity (safety) of protein therapeutic drug products, the majority of which are glycosylated [10-12]. Glycoanalysis is required for approval and licensing of protein therapeutic drugs and is used for quality control and process change monitoring [12-14]. With the eminent introduction of biosimilars into the marketplace, glycoanalysis will figure prominently in the determination of "sameness" of generic protein therapeutic drugs. The impact on efficacy and safety of a drug product due to a particular glycan structure or variation in that structure is unpredictable. Therefore, it is often required that all peaks detected in a glycoprofile should be controlled and specified down to a level dictated by the limit of detection (LOD) and/or limit of quantitation (LOQ) of the glycoanalysis techniques used. One consequence of this is that glycan species representing less than 5% of the total glycan may be required to be controlled and specified.

The requirement for this level of detailed characterization of the glycan content in biomanufacturing and other areas of research has lead to the development of sophisticated glycoanalysis techniques. Glycoanalysis is challenging for several reasons [1]. Unlike other areas of modern biology, glycosylation is not template driven and is therefore unpredictable. This is in stark contrast to the ability to predict the peptide sequence of a protein from its RNA and DNA sequences. Glycosylation is inherently heterogeneous with variation found in the identity, relative amounts, and linking of the sugar groups of the oligosaccharide examined [14, 15]. The chemical structures of sugar subunits can very similar, with no difference in molecular weight or charge. Even within a population of monoclonal antibodies produced using a cloned cell expression system, and highly monitored growth conditions, a heterogeneous glycan population can exist due to variation in expression levels [16, 17]. To complicate the analysis further, the glycans can be buried within the protein structure, as is the case for monoclonal antibodies, the largest class of protein therapeutics. Therefore the glycan is not easily accessible for recognition binding assays and sample preparatory steps must be taken to cleave off of the protein for further chemical and/or structural analysis.

The most common analytical techniques used for complex glycoanalysis include mass spectrometry [18-21], nuclear magnetic resonance spectroscopy [22-25], and separation techniques (e.g. high performance liquid chromatography and capillary electrophoresis) [26-28]. These methods are well-established and are able to provide detailed structural analysis, making them the most commonly used among expert glycoanalysis research centers [29-32]. Although the information gained by these analytical methods is detailed and rigorous, the time and expertise required to carry out these analyses does not lend themselves towards diagnostic or screening assays which find applications in quality control monitoring of biomanufactured samples, and biomarker screening for disease or infection. It has been suggested that complete structural detail is not required for monitoring, screening or diagnostic applications [33]. Ideally, accurate identification and quantitation of the terminal sugar

oligo(saccharides) is all that may be required for these types of applications. The selective, quantitative information that biosensors provide through the integrated transduction of a biological recognition event to a measureable signal are sufficient for this level of glycoanalysis, and may provide a solution to the need that has been expressed for high-throughput approaches for glycoanalysis that would be accessible to non-specialist laboratories [15, 33, 34].

2. Glyco-biosensors

Glyco-biosensors have recently made advances into this area of measurement science. Although sugar analysis by electrochemical methods (specifically we refer here to the large amount of literature devoted to glucose analysis by the glucose oxidase enzyme electrode [35-38]) has a long history, the extension to analysis of the glycan species of glycoconjugates (e.g. glycoproteins and glycopeptides) or cleaved glycan species are rare in comparison. There are several comprehensive reviews on the applications of biosensors for the study of glycans [33, 39] and carbohydrates in general [40, 41]. These reviews include a range of biosensors design where the transduction mechanisms include optical (e.g. surface plasmon resonance, SPR), piezoelectric (quartz crystal microbalance, QCM), electrochemical (e.g. electron impedance spectroscopy, EIS or pulsed amperometric detection, PAD), and μcantilever deflection.

2.1 Electrochemical Glyco-biosensors

Electrochemical transduction methods are attractive because they often do not require labeling of the glycan, the physical instrumentation required for electrochemical analysis is often very simple and inexpensive and the electrodes themselves can be made of different materials and in various sizes for specific applications. One of the most widely-used applications of electrochemical transduction methods for the analysis of carbohydrates is the coupling of an efficient separation technique, such as liquid chromatography (LC) or capillary electrophoresis (CE) with PAD. LC-PAD and CE-PAD systems have

demonstrated high selectivity for easily oxidized or reduced analytes, and limits of detection that rival fluorescence and mass spectrometric techniques [41]. These mature analytical systems have shown significant success over the past few decades. The application of LC, in particular high-performance anion exchange chromatography (HPAEC), and CE with PAD for the determination of oligosaccharide structure and the characterization of potential glycosylation sites in samples consisting of mixtures of oligosaccharides, glycoproteins, glycopeptides and glycoconjugates has been thoroughly reviewed by several groups [41-46].

Other electrochemical transduction methods used in glyco-biosensor design include: differential pulse voltammetry (DPV) [47-53], cyclic voltammetry (CV) [49, 53, 54], electrochemical impedance spectroscopy[52, 53, 55-64], potentiometry [65, 66], and square wave voltammetry (SWV) [67, 68]. This review is focused specifically on biosensor technologies applied to glycoanalysis (i.e. a glyco-biosensor) that use electrochemical transduction mechanisms, unless otherwise noted [69, 70].

Glyco-biosensor designs can be quite complex, due to challenges associated with direct electrochemical analysis of glycans. Although carbohydrates are able to be oxidized using chemical agents, they do not commonly exhibit redox behavior [41]. Similarities in the chemical structure of sugars, demand the use of a selective binding agent or a separation technique [41]. The most recognized biosensor design is that of the enzyme-electrode applied to the detection of glucose. In this example, the carbohydrate (glucose) is the substrate for the enzyme (glucose oxidase) which is proximal to the electrode surface; the redox active product of the enzymatic reaction is measured at the electrode surface, and is proportional to the amount of glucose in solution. This technology was first introduced 50 years ago [35, 37] and there are several comprehensive articles that review the history of research in this field [38, 71-75].

The most common types of biosensor design, are presented in Figure 2. All of them use selective binding agents, the most common of which are lectins (carbohydrate binding proteins) as will

be discussed below, and a redox probe combined with one of the electrochemical transduction technique listed above, the most common being EIS and DPV. Typically when EIS is used (Figure 2a) the electrode (planar or modified with a nanomaterial coating) is modified with a binding agent (lectin), which imparts selectivity and affinity. Changes in the charge transfer resistance at the electrode in the presence of a redox couple (e.g. $Fe(CN)_6^{3-/4-}$) are monitored and interpreted as binding of glycans, glycoconjugates or cell surface carbohydrates. In the case of a lectin biosensor sandwich assay (Figure 2b), a surface bound lectin is used to selectively attract a glycan target to the electrode surface, and a second redox active lectin-conjugate is used to bind to the captured target. Lastly, in the case of cell surface carbohydrate analysis, the cell is often captured at an electrode surface (planar or modified with a nanomaterial coating) and a lectin-enzyme in the presence of substrate is used to selectively bind to cell surface carbohydrates and provide the electrochemical signature (Figure 2c). As will be seen in specific examples from the literature discussed below, nanomaterials are often incorporated into these biosensor designs either to increase the surface area and subsequently the signal generated at the electrode, or as a redox agent.

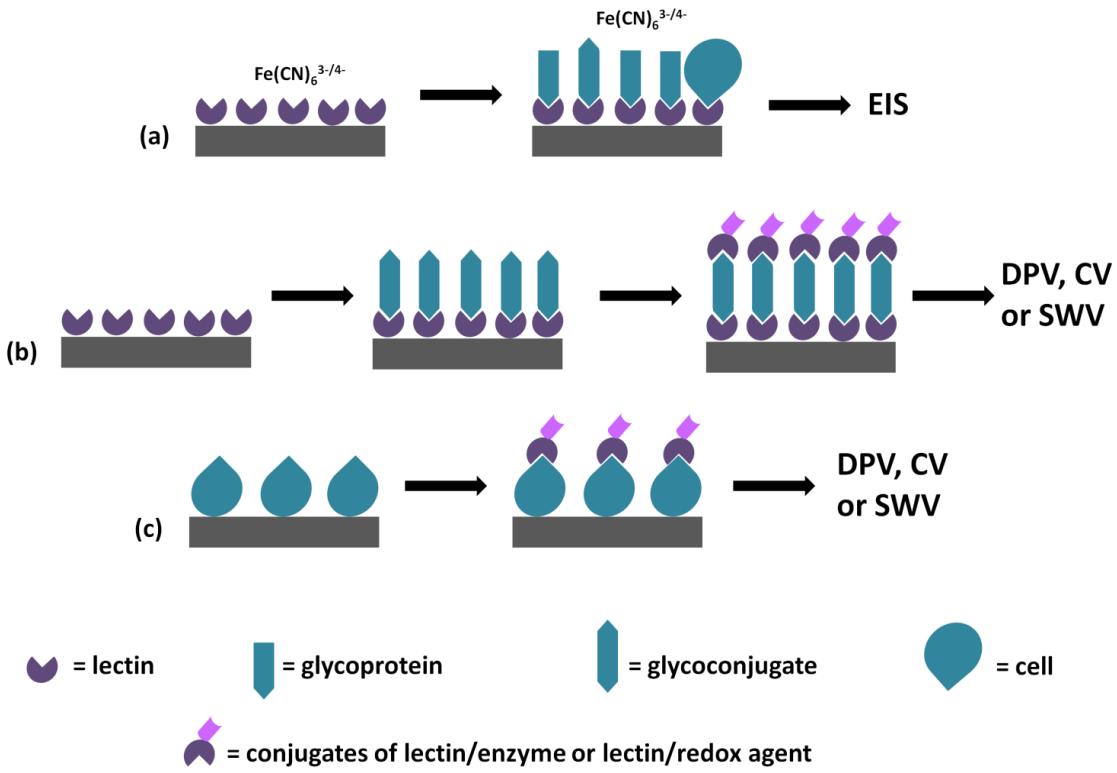

Figure 2. Schematic showing the most common types of electrochemical biosensors for glycan analysis

Lectins are by far the most commonly used selective binding elements used in glyco-biosensor measurements. Lectins are naturally occurring carbohydrate binding proteins that exhibit specificity dependent on the identity of the terminal sugar residue. Although it is the interaction of the lectin with the terminal sugar group that influences the specificity the greatest, quantitative differences in the affinity of lectins with various carbohydrate sequences, even in the case when the terminal sugar is the same, have been reported [76, 77]. Lectins are used in many areas of glycomic research including biomarker detection, clinical diagnostics, and the understanding of carbohydrate-protein interactions [34, 77-79].

A table of the lectins used and the sugar binding specificity for each instance surveyed here are listed in Table 1 [33].

Table 1: Lectins used in glyco-biosensor applications noted within this manuscript.

Lectin	Abbreviation	Major specificity	Reference(s)
Concanavalin A	Con A	Man, Glc, GlcNAc	[47, 49-53, 55, 56, 59-61, 63, 64, 80-82]
Peanut Agglutinin	PNA	Gal-β(1,3)-GalNAc (O-linked GalNAc)	[47, 56, 58, 67, 80]
Sambucus lectin	SNA I SNA II	Sialic acid-α(2,6) Gal/GalNAc	[50, 57, 58] [58]
Horse gram lectin	DBA	GalNAc	[47, 56, 80]
Wheat germ agglutinin	WGA	NeuAc/GlcNAc	[47, 56, 80]
Castor bean lectin	RCA	Gal	[55]
Lentil lectin	LCA	Man, Glc, GlcNAc	[82]
Maackia agglutinin	MAA	Sialic acid-α(2,3)	[57]
Cratylia mollis	Cramoll	Man, Glc	[62]

Con A, a mannose/glucose binding lectin, is most often used as a selective pull-down reagent, bringing glycans, glycoconjugates or cells to planar electrodes [57, 63] and to nanomaterial coated electrodes [56, 60, 61]. Con A has also been covalently bound to enzymes (e.g. horseradish peroxidase, HRP), creating a lectin-enzyme that binds to the glycan species of interest at the electrode, providing an enzymatically generated redox signal [47, 51]. Con A has also been used in lectin-glycan-lectin sandwich assays [50].

2.1.1. Glyco-biosensors based on Electrochemical Impedance Spectroscopy

Electrochemical impedance spectroscopy is an efficient, sensitive, rapid and inexpensive technique suitable for the characterization of transformations on electrode surfaces, and therefore, especially suited for the label-free transduction of biosensing events on electrodes [64]. EIS enables

rapid, label-free assays, through analysis of changes in the interfacial properties of the electrode interface associated with analyte binding. In the case of glycan analysis, EIS allows for the interrogation of lectin-glycan binding events by monitoring a change in the system impedance, or more specifically, a change in charge transfer resistance (R_{CT}) in the presence of a redox couple.

EIS measures the impedance of a system over a range of frequencies by applying to the system a small amplitude alternating current signal. EIS can be used to understand electrochemical reaction rates, and to describe interfaces due to its sensitivity to charge transfer processes that occur at the electrode/electrolyte interface. By fitting EIS data to an equivalent circuit, the value for R_{CT} (which models charge transfer across the interface), can be obtained. Binding events at the electrode surface would affect R_{CT}, due to the blocking effect that the immobilized molecules have on the charge transfer process; therefore, R_{CT} (or ΔR_{CT}) can be used as a detection parameter.

Electrochemical impedance spectroscopy, in conjunction with lectins, was used for the first time by La Belle and coworkers for the label-free detection of glycoconjugates on a chip based biosensor [58]. The plant lectins PNA and SNA were covalently attached to a gold electrode previously modified with mercaptohexadecanoic acid, and impedimetric measurements in the presence of the redox couple ferrocyanide/ferricyanide were used to demonstrate binding of artificial and natural glycoconjugates to the lectin-modified Au electrode. An “artificial glycoprotein” construct of Au nanoparticles (AuNP) encapsulated with TF-antigens (Galβ1-3GalNAc), as well as the glycoprotein asialofetuin (ASF), were rapidly detected on PNA-modified electrodes, whereas the glycoprotein fetuin (FET), the sialylated glycoform of ASF, was detected on SNA-modified electrodes with limits of detection in the fM concentration range.

The interaction between the glycoprotein ovalbumin and gold electrodes modified by the sol-gel method with conjugates of lectins and gold nanoparticles and with polyvinyl butyral (PVB) was studied by EIS in the presence of potassium ferro/ferricyanide in phosphate buffer [61]. The glucose/mannose-

specific lectins Con A and CramoLL were able to recognize ovalbumin, as evidenced by an increase in charge transfer resistance after addition of the glycoprotein to the electrode. Increases in the concentration of ovalbumin, from 25 μg/mL up to 200 μg/mL, resulted in increases in R_{CT}. These changes were confirmed by cyclic voltammetry.

The AuNP-lectin-PVB electrode prepared by Oliveira and coworkers [61] was also used to create an impedimetric biosensor to detect serum glycoproteins from patients infected by dengue fever (DF) [59]. A large increase in R_{CT} was obtained when the glycan portion of the glycoproteins present in the sera of patients infected with DF was allowed to interact with Con A in the modified Au electrode. A smaller increase in R_{CT} was observed when the serum of healthy patients was analyzed, demonstrating that the specific interaction between Con A and glycans can be used to discriminate between sera from healthy and DF patients. A subsequent report by the same group demonstrated that impedimetric measurements with the AuNP-Con A-PVB electrode can also be used to discriminate between the sera of patients infected by DF and dengue hemorrhagic fever (DHF), due to a higher expression of glycoproteins in the serum of DHF patients, which results in a larger increase in R_{CT} in comparison to the DF serum [60].

A similar EIS biosensor was fabricated by modifying Fe_3O_4 nanoparticles with the lectin Cramoll, mixing them with PVB and depositing them on a gold electrode [62]. The Fe_3O_4-Cramoll-PVB electrode was exposed to the glycoprotein fetuin and to sera from patients infected by different dengue serotypes, and increases in impedance were subsequently observed, indicating that the electrode can be used to sense lectin-glycan interactions. This biosensor effectively detected the presence of glycoproteins in just minutes, used small sample volumes, and was able to discriminate between different dengue serotypes.

The interaction between lectin-modified electrodes and the glycan components on cell walls has been used as the recognition principle to develop label-free impedimetric biosensors (Figure 3) to

analyze the glycan expression on living cells [56]. Furthermore, the specificity of the lectin-glycan interaction was also used to detect and identify bacteria [64]. For example, rapid, label-free electrochemical detection, identification and quantification of different bacteria were achieved by monitoring the impedimetric changes caused by the recognition between lectins and glycan components of bacteria walls. Nine different biotinylated lectins were mixed with the microorganisms, and subsequently deposited on the gold electrode for analysis. The biosensor detected, identified and quantified three different bacteria with a detection limit and linear range equal to or better than other electrochemical biosensors. Another advantage is that this impedimetric sensor was able to rapidly monitor the change in charge transfer resistance resulting from the interaction between the lectins and the bacteria at gold electrodes without any pre-concentration or pre-enrichment steps. The sensor showed the capability to discriminate between different types of bacteria by using multiplexed analysis with up to nine lectins (Figure 4).

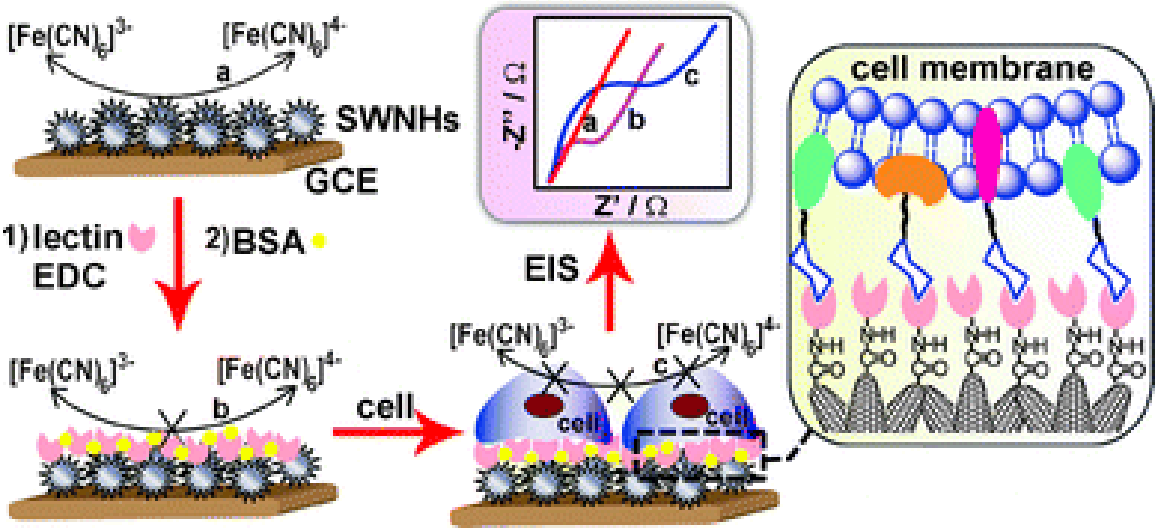

Figure 3. Schematic representation of the electrochemical label-free sensor for the analysis of glycan expression on cell surfaces. Permission requested from [56].

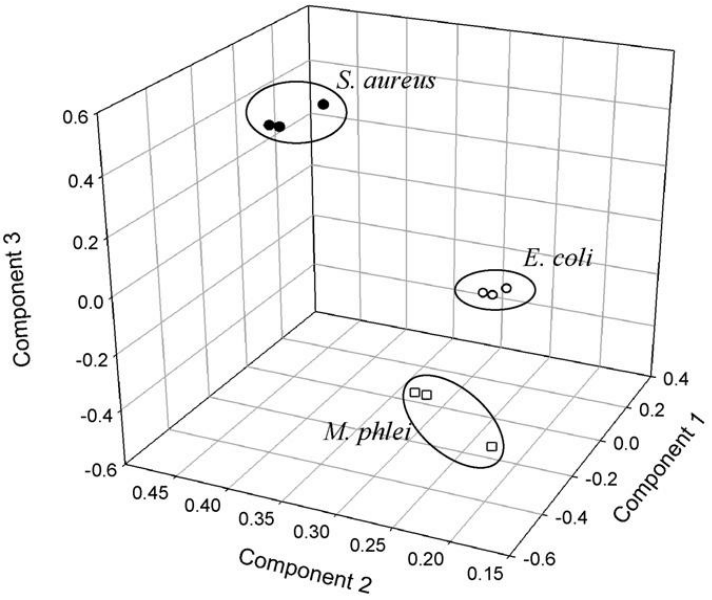

Figure 4. Pattern recognition plot obtained from impedimetric measurements using different lectins showing that EIS allows classification and distinction among different types of bacteria. Permission requested from [64].

Another lectin-based impedimetric biosensor was fabricated by Wan and coworkers for the rapid and label-free detection of sulfate-reducing bacteria (SRB) at Con A-modified Au electrodes [63]. Con A was covalently attached to a self-assembled monolayer of 11-mercaptoundecanoic acid on Au (Figure 5), and subsequently allowed to interact with SRB in order to determinate and monitor the bacterial growth by impedance measurements in $Fe(CN)_6^{3-/4-}$. Several parameters, including solution pH and incubation time, were optimized, and the concentration of the SRB was determined from the charge transfer resistance values obtained by EIS. Additionally, the specificity of the biosensor was investigated by analyzing different types of bacteria, and it was reported that equal concentrations of different species of bacteria induced different changes in the R_{CT} values. The impedimetric biosensor was used to obtain SRB growth curves similar to those obtained with the conventional and time-consuming most-probable number (MPN) method, thus demonstrating that EIS has great potential for the rapid, simple

and low-cost detection and monitoring of microbial populations. Furthermore, recent work by Xi *et al.* has shown that a gold electrode modified with lectins by the layer-by layer self-assembly technique can be used for the selective discrimination of gram-negative bacteria, gram-positive bacterium, fungus and mammalian cells by EIS [55].

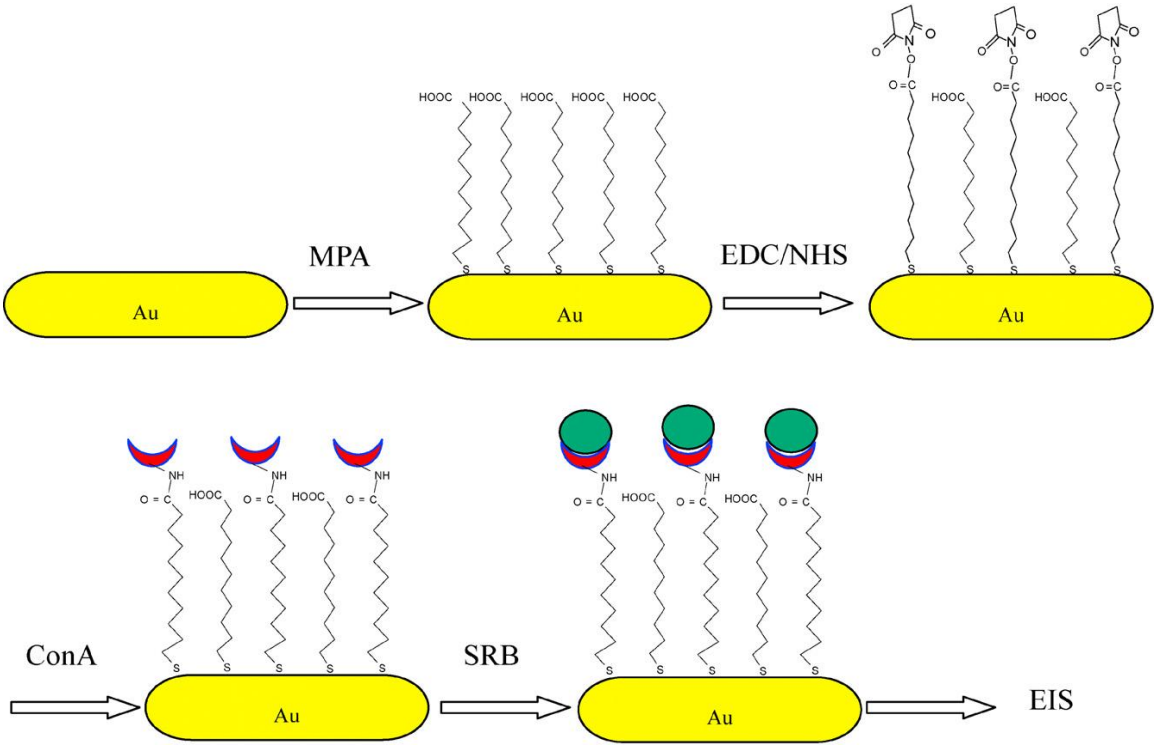

Figure 5. Scheme showing the steps required for the preparation of a lectin-based impedimetric biosensor for SRB detection. Permission requested from [63].

In related studies, EIS has been used as an effective technique to probe the sugar-binding specificity of lectins using carbohydrate-modified electrodes, composed of boron-doped diamond [83] and gold [84], among others. Furthermore, impedimetric measurements have elucidated the effect of changes in lectin conformation on glucose binding at platinum electrodes [82].

3. Applications: Biomarker and Cell Detection

Cell surface glycans play significant roles in many key biological processes, including cell differentiation, cell adhesion, cell recognition, and microbial pathogenesis [48]. Aberrant glycosylation patterns of cell surface carbohydrates have been linked to various diseases; thus it is essential to develop sensitive, reliable and high-throughput techniques to identify and detect cell surface carbohydrates. Due to their high sensitivity, low costs, simplicity, short assay time and ease of miniaturization, glyco-biosensors are a promising alternative for glycan biomarker discovery [57]. In the past few years, glyco-biosensors for cell surface glycans and potential biomarkers based on electrochemical impedance spectroscopy [57], potentiometry [66], and voltammetry [47, 53, 68] have been reported. Furthermore, in-situ imaging of membrane glycan motifs of human gastric carcinoma cells (BGC-823) has been performed by scanning electrochemical microscopy (SECM) [80].

One of the simplest glyco-biosensor designs for cell surface glycan monitoring combined the specificity of lectins-glycan binding with the electroactive properties of the ferrocenyl group [49]. Ferrocene monocarboxylic acid (FcCOOH) was covalently conjugated to Con A, and the electroactive species-lectin conjugate was allowed to interact with K562 cells. The cell-Fc-Con A conjugates did not diffuse freely to the electrode surface, which therefore induced a decrease in differential pulse voltammetry (DPV) peak current, as compared to free Fc-Con A. The magnitude of the decrease in current is proportional to the amount of K562 cells, as well as to the expression extent of mannose-presenting glycans on the cell surface. This simple approach was able to achieve cytosensing and cell surface glycan quantification.

DPV was also used for the sensitive detection and quantification of intestinal human colon adenocarcinoma (LS180) cells. The design of this novel glyco-biosensor was based on a competition between the specific binding of L-selectin to an aptamer, versus specific binding of L-selectin with glycans on the surface of LS180 cells. Binding of LS180 cells effectively blocks electron transfer between

the electroactive species (naphthoquinone) and the electrode, causing a decrease in the DPV peak current.

The principle of double layer capacitive measurements, was alternatively used by Nagaraj and coworkers to identify glycoform variants of fetuin and differences in glycosylation of protein extracts from a human pancreatic cancer cell line [57]. The sensor, named NanoMonitor, consisted of an array of gold electrodes on a silicon chip that were modified with lectins via biotin/streptavidin linker chemistry. Perturbations of the electrical double-layer occurred when glycans interacted with the lectins, and the perturbations were detected with impedimetric measurements. The sensor distinguished between different synthesized glycoforms of fetuin and recorded differences in glycosylation between protein extracts from human cancerous and normal pancreatic cells. In comparison to lectin-based ELISA assays, the NanoMonitor provided rapid, label-free analysis of glycoproteins with higher sensitivity (five orders of magnitude higher) and a broader dynamic range of glycoprotein concentrations.

Engineered nanomaterials, including nanoparticles [50, 66], nanotubes [47, 51, 53], quantum dots [67, 68, 81], and carbon nanohorns [52], have been used in conjunction with biological binding agents to fabricate glyco-biosensors for biomarker detection. For example, single-walled carbon nanotubes functionalized with a short peptide sequence (RGDS, an integrin binding sequence that inhibits integrin receptor function) were used to capture human leukemic K562 cells on a screen-printed carbon electrode (Figure 6) or BGC-823 human gastric carcinoma cells on a glassy carbon electrode [47, 51]. The glycans on the cell wall were used to capture lectins conjugated to horseradish peroxidase (HRP), and the characteristic electrochemical signal from HRP catalysis in a solution containing H_2O_2 and *o*-phenylenediamine was recorded by differential pulse voltammetry (DPV). DPV peak currents were used to quantify the amount of lectins captured, which is directly related to the quantity of glycans on the cell surface. The degree of glycan expression on the cancer cell surfaces and changes in glycan expression after drug treatment were determined with high sensitivity and reproducibility. A variation of

this assay showed enhanced sensitivity for the detection of K562 cell wall carbohydrates by combining single-walled carbon nanohorns and gold nanoparticles modified with Con A and HRP [52].

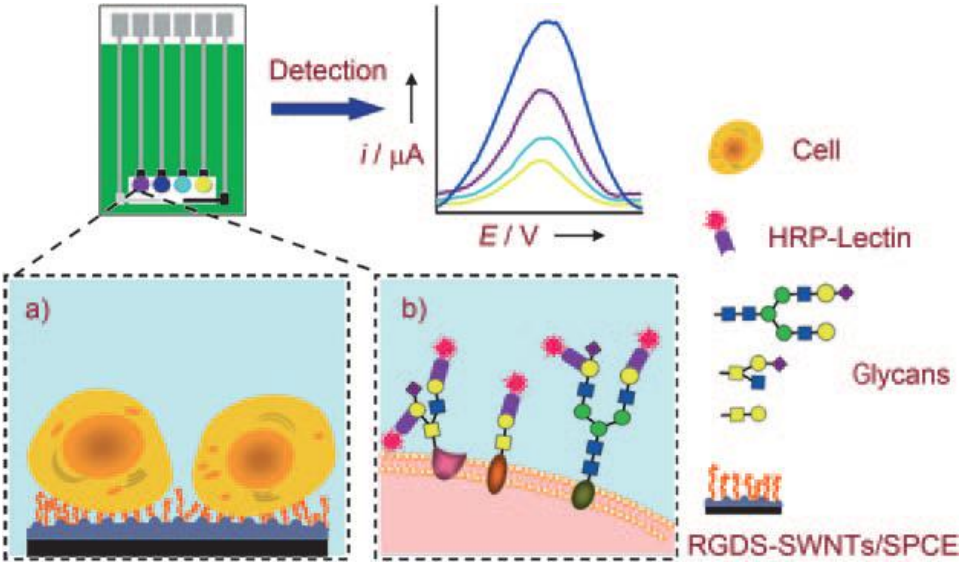

Figure 6. Schematic representation of the electrochemical cytosensor array for the analysis of carcinoma cell surface glycans. Permission requested from [47].

Zhang and coworkers also used DPV to design and implement an electrochemical enzyme-linked immunoassay for the detection of cell surface carbohydrates and an energy-dependent protein, P-glycoprotein, which can be found on tumor cells [53]. The HRP-catalyzed oxidation of thionine by H_2O_2 was monitored by cyclic voltammetry, EIS and DPV at a glass carbon electrode modified with nitrogen-doped carbon nanotubes, gold nanoparticles and Con A (Figure 7). The specific binding between Con A and cell surface mannosyl groups was used to capture human epithelial carcinoma cells. Electrocatalytic peak currents obtained from DPV measurements were correlated to the amount of glycans present on the cell surface. The designed cytosensor, while complex, showed good stability and reproducibility, and a wide linear range and low detection limit for the quantification of P-glycoprotein and cell surface carbohydrates.

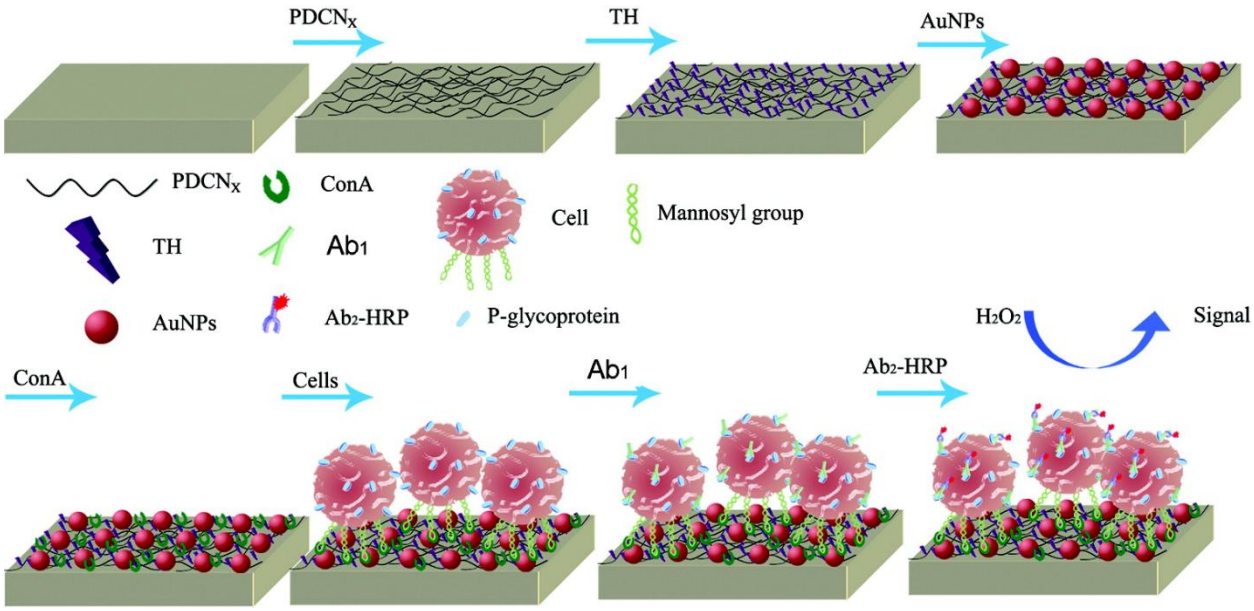

Figure 7. Schematic representation of the fabrication of the electrochemical enzyme-linked immunoassay for the detection of cell surface glycans. Permission requested from [53].

Alternatively, the potential shift that occurs when an electrode reacts with an ovarian tumor marker was used as the basis for the design of a potentiometric immunoassay for carbohydrate antigen-125 (CA125) [66]. Multifunctional magnetic beads, synthesized using magnetic Fe_3O_4 nanoparticles and poly(amidoamine) dendrimer, were used as an affinity support for the immobilization of anti-CA125, and with the aid of a magnet, were attached on a carbon paste electrode. The potential shift recorded after CA125 was bound to the electrode allowed for the simple, rapid and sensitive electrochemical detection of the CA125 tumor marker. Detection of the CA125 mucin-like glycoprotein was also achieved by a sandwich-type electrochemical immunoassay using anti-CA125-coated magnetic beads for CA125 capture and immobilization on the electrode, and anti-CA125-coated nanosilica particles doped with HRP and thionine for signal enhancement [54].

Another sandwich-type immunoassay was developed by Baoxiang *et al.* to detect the carbohydrate antigen 19-9 (CA 19-9), a marker for pancreatic cancer [68]. Conjugates of ZnO quantum

dots and the antibody for CA 19-9 were used to create a sandwich structure through the immunoreactions with CA 19-9 and a monolayer of CA 19-9 antibody on a silicon wafer. The ZnO quantum dots linked to the substrate were dissolved in acidic media, and the solution containing Zn^{2+} was accumulated at the electrode and analyzed by square wave stripping voltammetry (SWSV). This reusable immunosensor presented high sensitivity, stability, selectivity and good reproducibility for the detection of the pancreatic cancer marker CA 19-9. Furthermore, SWSV in conjunction with CdS or CdTe quantum dots has also been used to develop competitive assays for the analysis of K562 cell surface carbohydrates [81], and for the detection of the cancer associated T-antigen [67].

The sandwich format was also used to fabricate a lectin-based sensor for the analysis of sialic acid potential biomarkers for human lung, liver, and prostate cancer [50]. A composite film of gold nanoparticles and multi-walled carbon nanotubes was used for the immobilization of lectins (Figure 8). The sandwich-type system was formed by the binding of the glycans on the cell wall surface to the lectin-modified electrode, followed by the attachment of gold nanoparticles labeled with lectins and the electroactive species thionine to the immobilized cells. The electrochemical signal of thionine was correlated to the carbohydrate expression levels on the cells, and indirectly to the amount of bound cells, therefore allowing cell quantification. A label-free, potentiometric sensor based on the interaction between phenylboronic acid (PBA) and 1,2- or 1,3-diols, was applied to the analysis of altered sialic acid expression on erythrocyte as a model for diabetes diagnosis [65]. The previously described glyco-biosensors were capable of detecting enhanced expression of sialic acid in cancer cells as compared to normal ones, and differences in sialic acid content in erythrocytes, thus demonstrating that sialic acid could serve as a potential biomarker for different types of cancer, as well as for diabetes mellitus.

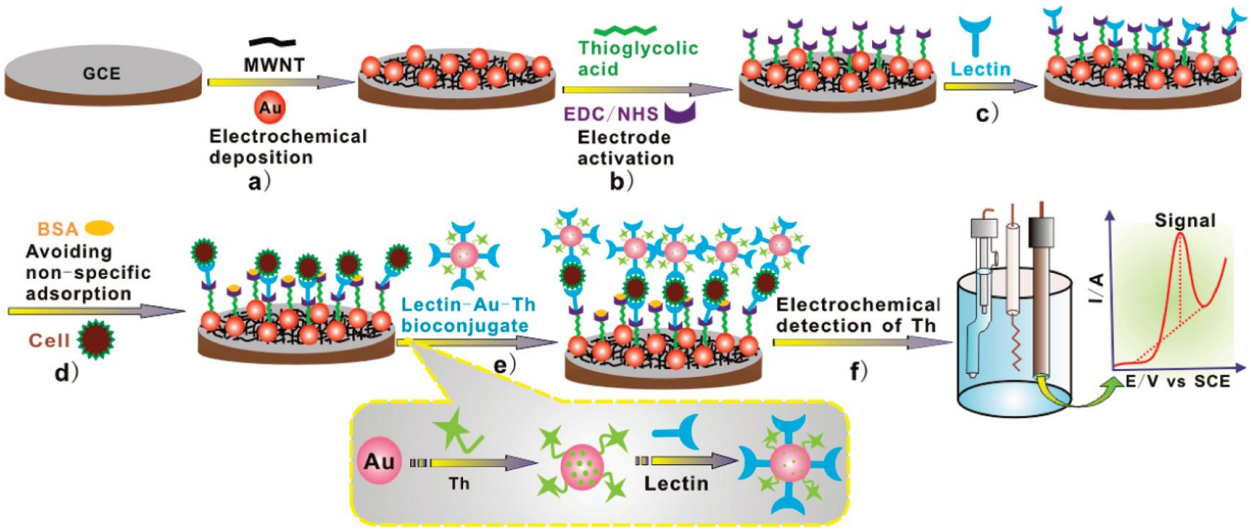

Figure 8. Schematic of the sandwich-type lectin based sensor for electrochemical analysis of glycan expression on cells. Permission requested from [50].

4. Summary

Glyco-biosensors offer simple, rapid, sensitive and economical approaches to the measurement needs associated with desired rapid glycan analysis for biomarker detection, cancer and disease diagnostics and bioprocess monitoring of therapeutic glycoproteins. The examples discussed here are generally singular in occurrence, but the successful application of a variety of electrochemical transduction methods, combined with novel redox probes speak to the compatibility of biosensors with glycoanalysis. The high sensitivity and broad dynamic range reported for the NanoMonitor system is one example of how a well engineered glyco-biosensor can offer a rapid and label-free alternative to glycan monitoring by traditional lectin-based ELISA assays. As new information about glycan structure and function is gleaned from glycomics researchers, new glyco -biosensors can be developed. In addition to lectin binding agents, the integration of aptamer and antibody binding agents into electrochemical glyco-biosensors may improve the specificity of these assays. Further advances in the development of these types of glyco-biosensors will help to effectively

transfer the knowledge gained from specialist glycoanalysis research facilities into practical assays for high-throughput analysis that can be used in clinical and biomanufacturing settings.

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